

Rotaxanating metallo-supramolecular nano-cylinder helicates to switch DNA junction binding

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Rotaxanating Metallo-supramolecular Nano-cylinder Helicates to Switch DNA Junction Binding

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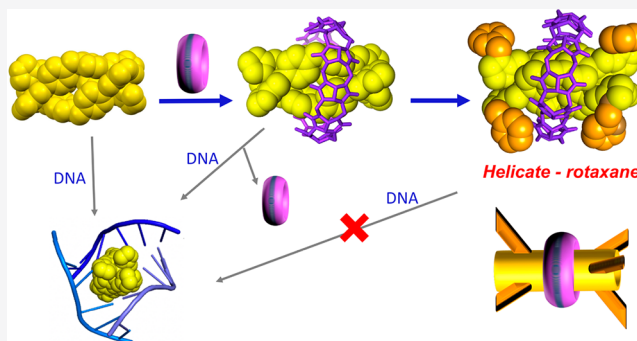


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ABSTRACT: A class of rotaxane is created, not by encapsulating a conventional linear thread, but rather by wrapping a large cucurbit[10]uril macrocycle about a three-dimensional, cylindrical, nanosized, self-assembled supramolecular helicate as the axle. The resulting pseudo-rotaxane is readily converted into a proper interlocked rotaxane by adding branch points to the helicate strands that form the surface of the cylinder (like branches and roots on a tree trunk). The supramolecular cylinder that forms the axle is itself a member of a unique and remarkable class of helicate metallo-drugs that bind Y-shaped DNA junction structures and induce cell death. While pseudo-rotaxanation does not modify the DNA-binding properties, proper, mechanically-interlocked rotaxanation transforms the DNA-binding and biological activity of the cylinder. The ability of the cylinder to de-thread from the rotaxane (and thus to bind DNA junction structures) is controlled by the extent of branching: fully-branched cylinders are locked inside the cucurbit[10]uril macrocycle, while cylinders with incomplete branch points can de-thread from the rotaxane in response to competitor guests. The number of branch points can thus afford kinetic control over the drug de-threading and release.



INTRODUCTION

Drawing on nature for inspiration, supramolecular chemistry has created a variety of complex, often exotic, nanoscale structures. Mechanically linked molecules have been of particular interest, and rotaxanes have been formed by threading molecular strands through a variety of different classes of macrocycle.^{1–3} Applications of rotaxanes have ranged from protecting peptides from rapid enzymatic degradation, to creating materials with switchable surface properties.^{1–8} Yet the threads used, to date, have been principally linear, 1-dimensional or 2-dimensional, organic, covalent molecules.

An alternative, but equally exciting, aspect of supramolecular chemistry, has been to take the designs and lessons of supramolecular chemistry and to apply them back to the biology that provided the initial inspiration for the field. Indeed self-assembled metallo-supramolecular helicates^{9,10} are enjoying a resurgence of interest for their application in biology.^{11–20} They offer quite large nanoscale surfaces of defined topography, of comparable scale to key structures found in biomolecules. We have used these external surfaces to access unique DNA- and RNA-recognition properties,^{21–23} demonstrating with X-ray crystal structures the remarkable binding of cylindrical metallo-helicates in the heart of DNA

and RNA 3-way junction structures,^{24–28} where the nucleic acid bases form perfectly stacked aromatic interactions²⁹ with the external aromatic surfaces of the metallo-helicates. Nucleic acid junction structures are exciting targets found both in biology and nucleic acid nanoscience.^{30–35} Our junction-binding cylinders arrest the proliferation of cancer cells and the HIV virus.^{36–39}

Given that the supramolecular nano-cylinder helicates thread so beautifully *into and through* junctions and bulges in nucleic acids, we were intrigued to see whether we could push the boundaries of supramolecular assembly to similarly thread such self-assembled nanoscale metallo-supramolecular helicates through *synthetic* macrocycles to create new types of rotaxanes and pseudo-rotaxanes in which the thread was not a simple chain but a three dimensional, self-assembled, metallo-cylinder.

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Since the cylinders recognize DNA-junction structures through their external surfaces, we anticipated that wrapping another molecule around this region of the cylinder might also interfere with (and ultimately control) the DNA-binding. Rotaxanation in DNA-binding agents has not been previously explored.

We report now, for the first time, the wrapping of a synthetic macrocycle around the external surface of a self-assembled metallo-supramolecular nanostructure to create a pseudo-rotaxane, subsequent conversion to a proper (mechanically-interlocked) [2]-rotaxane, and the effect of rotaxanation on the biomolecular recognition and biological properties of the metallo-supramolecular cylinders. Examples of larger threads in rotaxanes include pseudo-rotaxanes created by clipping macrocycles around carbon nanotubes,^{40,41} a rotaxane with a spherical fullerene in the center,⁴² and DNA rotaxanes,⁴³ but this work is distinct in representing (to the best of our knowledge) the first example of a proper interlocked rotaxane assembled around a three-dimensional metal-assembled supramolecular helicate as axle. An interesting report of rotaxanation of a helical peptide has also appeared, but in that case the small rotaxane ring wraps just around its linear chain (primary structure) rather than encapsulating a helical secondary structure.⁴⁴ The large three-dimensional cylindrical surface that our metallo-supramolecular helicate axle affords also brings another opportunity: rather than attaching a branched organic unit at each end of the thread as a stopper, we now clip unbranched units onto each thread of the helicate axle exploiting the metal-coordination and supramolecular structure to achieve the branching effect which mechanically constrains the ring. We show that the rotaxanation allows us to switch the DNA-binding properties and thus the biological activity of the cylinder.

RESULTS AND DISCUSSION

The first challenge was to identify a suitable “ring” that might be wrapped around these metallo-supramolecular helicates. They are tetracations formed by wrapping three organic ligand strands about two metal centers (Figure 1).¹¹ They are cylindrical in shape, around 2 nm in length, and 1 nm in diameter—much greater than the dimensions normally associated with rotaxanation or encapsulation—but we identified that cucurbit[10]uril (Figure 1) might offer a cavity of suitable dimensions.^{45,46}

Cucurbit[*n*]urils (CB[*n*]) are a class of macrocycles that are attracting much attention for both host–guest chemistry and rotaxanation studies.^{47–52} They are pumpkin-shaped molecules with a rigid hydrophobic cavity flanked by two identical openings (portals) whose rims are each lined with *n* carbonyl groups. CB[5,6,7] are ideal sizes for traditional small organics and CB[6,7,8] have been used with organic, linear threads and chains to create rotaxanes.⁴⁸ Other applications have included using the host guest chemistry to attach proteins to surfaces or fluorophores, and for drug delivery.^{47,53} The largest of the class, with an untwisted cavity, is CB[10] (Fig. 1) which can accommodate multiple or larger molecules^{45,46} as demonstrated by its use to capture both a Stoddart “blue-box” (bis-bipyridinium) macrocycle and its methoxybenzene guest to create a Russian-doll style assembly.⁵⁴ Binding interactions include hydrophobic effects in the heart of the cavity and ion-dipole interactions at the rims.^{45–54}

While lower order cucurbiturils have relatively rigid cavities, CB[10] is more flexible. It is observed to be ellipsoidal in its crystal structure (cavity diameter 1.13–1.24 nm; portal

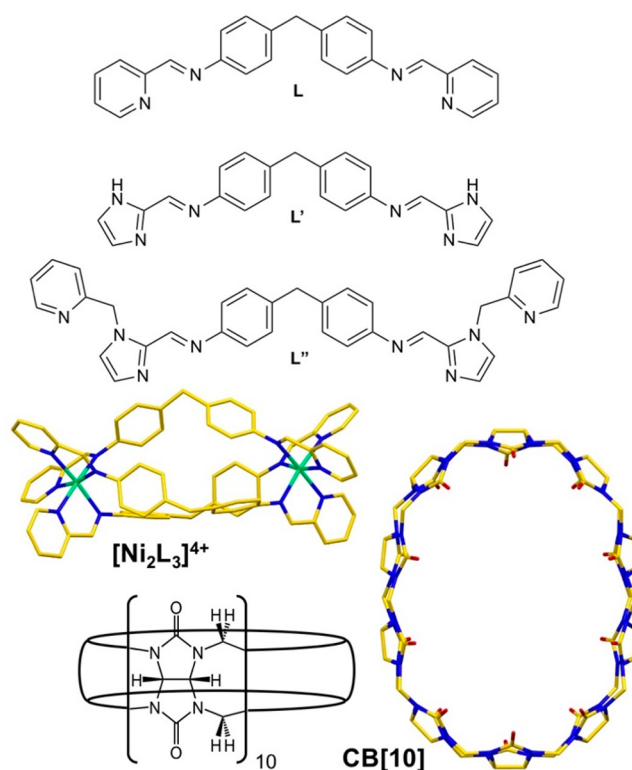


Figure 1. Ligands used in this study together with the structure of the [Ni₂L₃]⁴⁺ cylinder (CSD-NITBIB) and cucurbit[10]uril (from CSD-LAZPIM) and illustrating the flexibility and potential ellipsoidal distortions CB[10] can accommodate in response to guests).

diameter 0.95–1.06 nm) in complex with 4,6-bis(4-(ammoniomethyl)phenylamino)-1,3,5-triazene-2(1H)-one,^{45,46} and it is observed to also complex platinum terpyridyls⁵⁵ and porphyrins⁵⁶ which implies further distortion is possible. The previous use of CB[10] for rotaxanation is restricted to a report of a kinetic rotaxane—a pseudo-rotaxane in which the thread is encapsulated at room temperature but can enter and leave at elevated temperatures.⁵⁷

To initiate our studies we first explored whether the supramolecular cylinders would bind to CB[10] to give a tube threaded through a ring. CB[10] itself is an insoluble compound, but rapidly dissolves on addition of an aqueous solution of [M₂L₃]⁴⁺ cylinders (M = Fe, Ru, Ni). Electrospray mass spectrometry shows peaks corresponding to [M₂L₃·CB10]⁴⁺ (Figure S1 of the Supporting Information, SI) and proton NMR spectroscopy of [Fe₂L₃]⁴⁺ with CB[10] confirms formation of a 1:1 complex (Figure 2). DOSY NMR reveals that a single species is formed, of greater hydrodynamic radius than the free cylinder, and involving both cylinder and CB[10]. The cylinder retains its two-fold and three-fold symmetry in the complex. The proton NMR resonances corresponding to the central CH₂ of the cylinder and the adjacent phenylene protons experience upfield shifts, consistent with a shielded location in the CB[10] cavity, while the imine proton and the pyridine H₃ are shifted downfield, consistent with encountering the carbonyls at the rim of the macrocycle. NOEs are observed from these two cylinder protons (H_{im} and H₃) to the CH₂ proton on the rim of the CB[10] macrocycle (Figure S2). The NMR is unambiguous confirmation that the cylinder sits symmetrically (on the NMR time scale) in the center of the macrocycle threading through from one side to the other as a

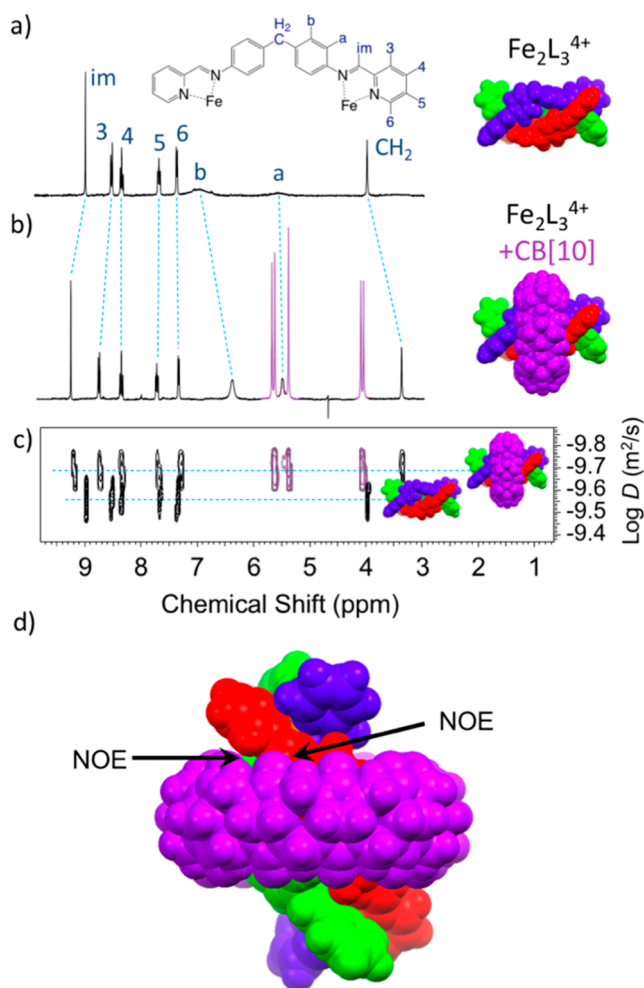


Figure 2. 500 MHz ^1H NMR spectra of the $[\text{Fe}_2\text{L}_3]^{4+}$ cylinder (0.5 mM) (a) alone and (b) with 1 equiv of CB[10] in D_2O illustrating the shifts caused by binding. (c) DOSY NMR spectra (stacked) showing the change in diffusion coefficient on binding consistent with formation of a single species of increased hydrodynamic radius. (d) DFT model of the cylinder + CB[10] also showing the experimentally observed NOEs from cylinder H-im & H-3 to a CH_2 proton on the rim of the CB[10].

pseudo-rotaxane, with the macrocycle sitting over the central diphenylmethanes of the cylinder as desired. To retain its three-fold symmetry within the 10-fold symmetric CB[10], the cylinder must be rapidly spinning or rocking about the metal–metal axis while within the macrocycle. DFT calculations and MD simulations reproduce this solution structure, with the DFT structure showing axial deviation between the host and guest main symmetry axes at the energy minimum (Figure 2d) and the MD showing the rotation. The binding of the cylinder places the diphenylmethane units within the hydrophobic cavity and does not leave space for solvent to co-bind in the cavity—water exclusion is understood to be an important part of CB[*n*] guest binding.^{45–54} The positively-polarized imino and pyridyl CH protons are located close to the oxygen-rich rim (as indicated by the NOEs to the CH_2 at the rim) though individual $\text{CH}\cdots\text{O}$ interactions must be transient since the cylinder is rotating rapidly. Use of a small excess of cylinder (Figure S3) in the NMR allows free and bound cylinder peaks to be observed indicating that exchange is slow on the NMR time scale, however on mixing $[\text{Fe}_2\text{L}_3]^{4+}$ with $[\text{Ni}_2\text{L}_3\cdot\text{CB10}]^{4+}$

and immediately injecting into an electrospray mass spectrometer a mixture of $[\text{Fe}_2\text{L}_3\cdot\text{CB10}]^{4+}$ and $[\text{Ni}_2\text{L}_3\cdot\text{CB10}]^{4+}$ was observed, the ratio of which remained unchanged over a further hour of sampling (Figure S4). Together these observations indicate that the exchange takes place over a time scale of milliseconds to seconds, which is consistent with measurements on other cucurbiturils with different guests.⁵⁸ The proton NMR spectrum of $[\text{Ru}_2\text{L}_3]^{4+}$ with CB[10] shows analogous upfield and downfield shifts in the resonances and the paramagnetically shifted proton NMR of $[\text{Ni}_2\text{L}_3]^{4+}$ with CB[10] also shows the binding (Figures S5 and S6).

While the ruthenium(II) and nickel(II) $[\text{M}_2\text{L}_3]^{4+}$ cylinders have excellent stability, the iron(II) cylinders can experience some hydrolytic degradation over long periods,^{11,37} although they are stabilized by binding to DNA.^{21,22} Encapsulation of the cylinder within the CB[10] macrocycle similarly enhances the cylinder stability; an experiment with 0.9 equiv of CB[10] revealed no degradation of the bound cylinder in aqueous solution over a period of 194 days (Figure S7). The insolubility of unbound CB[10] prevents accurate measurements of binding affinity with guests, however an NMR dilution experiment showed no unbound cylinder (Figure S8) implying the binding constant is greater than 10^7 M^{-1} , and NMR competition studies show that the cylinder binds more strongly than both methyl viologen and memantine hydrochloride.

To explore the effect of the encapsulation on the cylinder's DNA-recognition properties we investigated the binding of the nickel cylinder-CB[10] complex (as representative of the three complexes) to DNA 3-way junctions (3WJ)^{24–28,30,31} using gel electrophoresis. The assay (Figure 3a) uses a 3WJ formed from three different complementary DNA strands. The 3WJ structure is (entropically) unstable at room temperature in absence of the cylinder, and the DNA exists as three single strands. When the cylinder binds, the 3WJ is stabilized and observed in the gel. Radiolabelling one strand allows a simple on/off gel-shift binding assay that is readily visualized. Figure 3a shows that the cylinder-CB[10] complex stabilizes the 3WJ and the effect of the cylinder-CB[10] complex is the same as the cylinder alone, although higher loadings are required. This indicates that the cylinder can leave the CB[10] cavity and bind to the anionic DNA 3WJ cavity; while binding of an intact cylinder-CB[10] complex to the 3WJ cannot be excluded this would be expected to give a different gel shift. The cylinder binding to the 3WJ is in competition with the binding to the CB[10]; adding small amounts of solid CB[10] or of the soluble methylviologen CB[10] complex to the pseudorotaxane leads to small reductions in 3WJ formation (Figure S9). In these experiments, the binding energy to the 3WJ also incorporates the energetic cost of assembling the 3WJ from the DNA single strands. Given that the 3WJ is not observed in absence of cylinder at room temperature, the binding constant of cylinder to the 3WJ structure itself must be (significantly) greater than that to CB[10].

We also studied the binding to calf-thymus DNA (a polymeric genomic DNA), using circular and flow linear dichroism spectroscopies (Figure 3b,c). The effects are again the same as the free cylinder binding: The circular dichroism confirms binding of cylinder (an induced CD signal is observed in the cylinder spectroscopy between 300–400 nm) and retention of a B-DNA structure (typical signature between 200–300 nm). The CD spectra are the same as those observed with cylinder alone (Figure S10), although at high loading some scattering is observed consistent with release of CB[10]

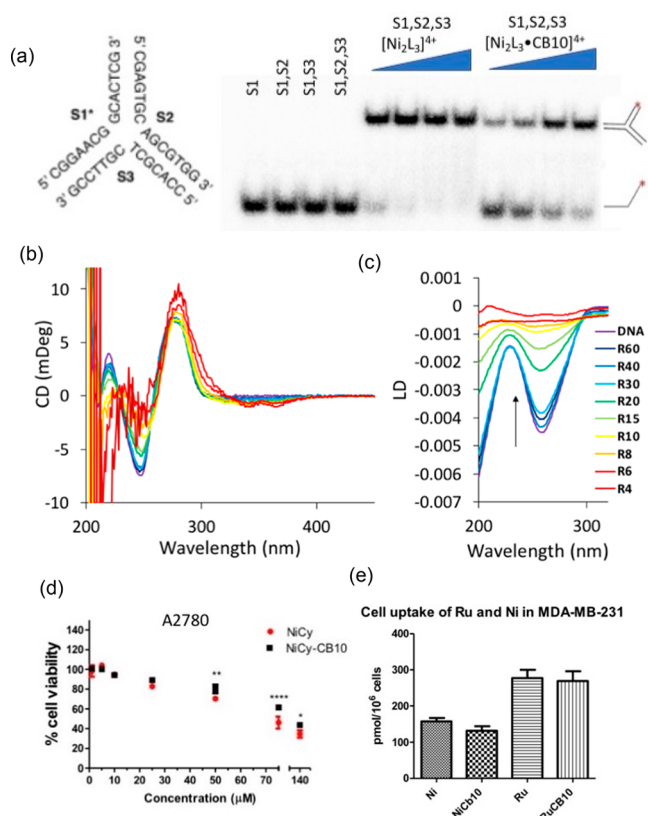


Figure 3. (a) PAGE gel showing that the $[\text{Ni}_2\text{L}_3\cdot\text{CB10}]^{4+}$ complex stabilizes DNA 3WJ in the same way as $[\text{M}_2\text{L}_3]^{4+}$ cylinders alone. (* = radiolabel) (b) CD and (c) LD spectra of ct-DNA (100 μM DNA concentration; 10 mM sodium chloride; 1 mM sodium cacodylate) with $[\text{Ni}_2\text{L}_3\cdot\text{CB10}]^{4+}$. *R* values represent DNA base pairs/complex. The spectra show binding and DNA coiling and importantly are analogous to those obtained with free $[\text{Ni}_2\text{L}_3]^{4+}$ (Figure S10) (d) MTT cell survival assay (72 h treatment of A2780 cells), demonstrating that $[\text{Ni}_2\text{L}_3\cdot\text{CB10}]^{4+}$ and $[\text{Ni}_2\text{L}_3]^{4+}$ have similar effects on cell survival (triplicate independent repeats; $\pm\text{SD}$; two way ANOVA analyses). (e) Cell uptake studies (triplicate; $\pm\text{SD}$) demonstrating that CB[10] does not affect the cell uptake. The greater uptake of the Ru (over Ni) cylinder is consistent with previous observations in 1G5 (Jurkat T-) cells.³⁷

which (uncomplexed) will be insoluble. The flow linear dichroism experiments probe the orientation of ct-DNA in a Couette flow cell, with the magnitude of the peak at 260 nm reflecting the orientation.^{21,22} Cylinders are known to coil ct-DNA (leading to a loss of orientation);^{21,22} the spectra on addition of the cylinder-CB[10] complex confirm the same DNA-coiling effects and are analogous to those observed with free cylinder (Figure S10). We conclude that while the CB[10] does bind the cylinder in solution to form the pseudo-rotaxane, the cylinder would prefer to bind to anionic DNA. For the 3WJ, which is not pre-formed in solution, the extra energy of forming the structure means that there is competition between binding CB[10] or 3WJ. When presented with a polymeric DNA anion, the cylinder leaves the CB[10] cavity and binds its preferred partner. Preferential binding to the anionic DNA over the neutral CB[10] cavity is unsurprising, given the anticipated electrostatic contribution to that binding energy, though it contrasts with spermine that preferentially binds CB[7] over DNA.⁵⁹

Consistent with the in vitro DNA binding, when we treat cells (A2780 ovarian and MDA-MB-231 breast cancer lines) with the cylinder-CB[10] complex, we observe very similar inhibitions of cell proliferation (as assessed by an MTT assay) to those with the corresponding free cylinder (Figures 3d and S11). There is a very slight enhancement in activity for the ruthenium cylinder but a slight drop in activity for the iron and nickel cylinders. Uptake studies (assessed by ICP-MS) indicate that the encapsulation inside CB[10] has little effect on the extent of overall uptake (Figure 3e). Cucurbiturils are frequently used as drug delivery agents and platinum metallo-drugs (e.g., oxaliplatin) encapsulated inside cucurbit[7]uril show some enhanced effects^{60–65} due to better uptake and/or pharmacology (less degradation and side reactions with thiols). The lack of dramatic enhancement for the cylinder perhaps reflects the already excellent cell uptake of cylinders^{36,37} and the lack of competing side reactions. The DNA-binding studies indicate that whether the cylinders have entered alone or in complex with CB[10], their effects when they reach the target DNA are expected to be the same. Consistent with this, a dinuclear ruthenium complex (from Keene, Collins et al.)^{66,67} that also binds non-covalently to DNA, and that can partially

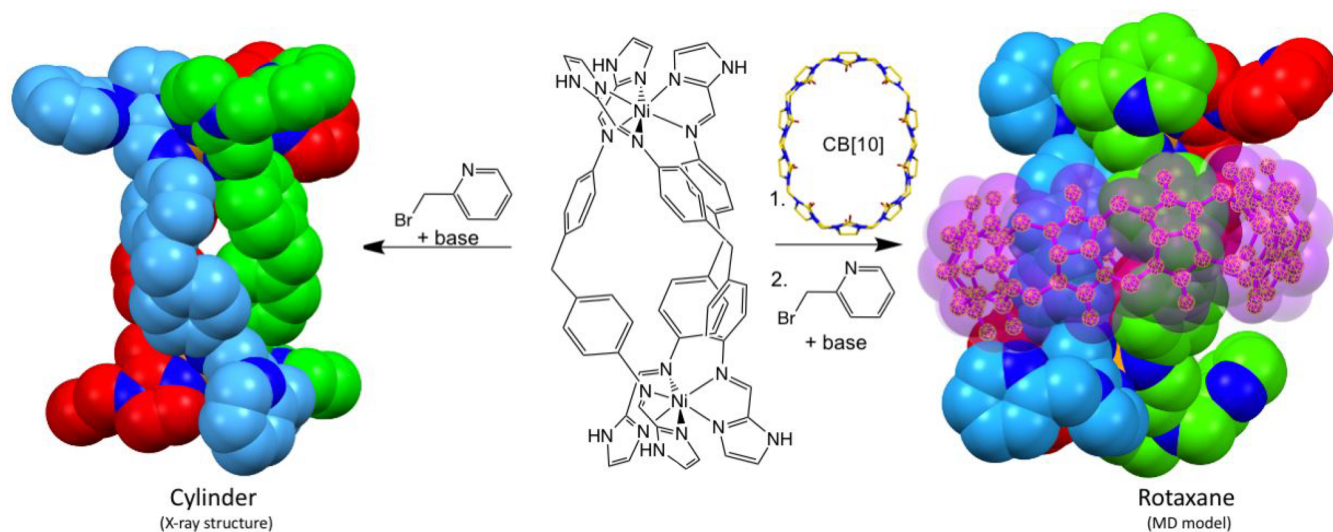


Figure 4. Our synthetic strategy to create a rotaxanated cylinder.

insert (but is too large to thread) into CB[10], similarly showed only small effects of complexation on its in cellulo behavior (though the DNA-binding of that agent is different and the complex only mildly cytotoxic).

Having established that pseudo-rotaxation with CB[10] was readily achieved and that it placed the macrocycle in the correct position over the central section of the cylinder but that it had little effect on the biological properties due to competitive de-threading, we next explored if we could lock the macrocycle onto the cylinder as a proper rotaxane. Traditional rotaxanes are formed by attaching two bulky stoppers, one at each end of the thread. By using a 3-dimensional helicate as the axle, we open up an alternative possibility to create the branching needed to lock the ring onto the structure, by attaching multiple (smaller) groups that protrude out of the cylindrical surface at the ends of the helicate axle. To explore this, we modified the design of the cylinder, replacing the pyridines with 2-imidazole units. The 2-imidazole offers a position to which we can add further functionalization, through alkylation of the non-coordinated nitrogen in the imidazole ring, and we selected 2-picoyl as the alkylating group. Thus, we introduce three branch points onto the cylinder surface at each end. This new design approach is shown in Figure 4.

We first prepared two non-rotaxanated cylinders, $[\text{Ni}_2\text{L}'_3]^{4+}$ and $[\text{Ni}_2\text{L}''_3]^{4+}$, analogous to the parent cylinders but bearing the 2-imidazole and 2-imidazole-*N*-picoyl groups in place of the pyridines. They are readily prepared from the condensation of the 2-imidazole and 2-imidazole-*N*-picoyl carboxaldehydes with bis-1,4-diaminophenylmethane and subsequent coordination to nickel(II). While the corresponding iron(II) complexes were also prepared, $[\text{Fe}_2\text{L}'_3]^{4+}$ had a lower solution stability than the corresponding nickel(II) complex and so we focused our studies on the nickel complexes. The X-ray crystal structures of the $[\text{M}_2\text{L}'_3]^{4+}$ cations ($\text{M} = \text{Ni}, \text{Fe}$) were obtained (as the hexafluorophosphate salts) and confirmed the expected structure (Figures 4 and S12), with the retention of the triple-helical cylindrical structure, the retention of the central core of the cylinder structure which is crucial for its binding to both DNA 3-way junctions and CB[10], and now the introduction of the picoyl units sticking out of the helix and in such a position that they should not interfere with the CB[10] binding site but would potentially sterically prevent it from threading/dethreading.

We then explored whether we could post-synthetically transform $[\text{Ni}_2\text{L}'_3]^{4+}$ into the alkylated $[\text{Ni}_2\text{L}''_3]^{4+}$ by reaction with alkylating agent 2-(bromomethyl)pyridine and Hunig's base in acetonitrile; heating at reflux overnight afforded the desired hexa-alkylated product, $[\text{Ni}_2\text{L}''_3]^{4+}$, as confirmed by mass spectrometry and (paramagnetic) proton NMR spectroscopy. Small amounts of the penta-functionalized cylinder were also observed by mass spectrometry. To access the rotaxane, we then repeated the same reaction in the presence of CB[10]. While acetonitrile did not prove to be a suitable solvent, reaction in aqueous methanol at reflux overnight, afforded the rotaxane as a cream precipitate in ~60% isolated yield. The mass spectrum (Figures 5 and S14) shows a dominant peak corresponding to $[\text{Ni}_2\text{L}''_3\cdot\text{CB10}]^{4+}$ (correct mass and isotope distribution). Smaller peaks corresponding to penta-alkylated rotaxanes are also observed but only a tiny trace peak corresponding to un-rotaxanated $[\text{Ni}_2\text{L}''_3]^{4+}$ indicating that the rotaxane (as opposed to a pseudo-rotaxane)

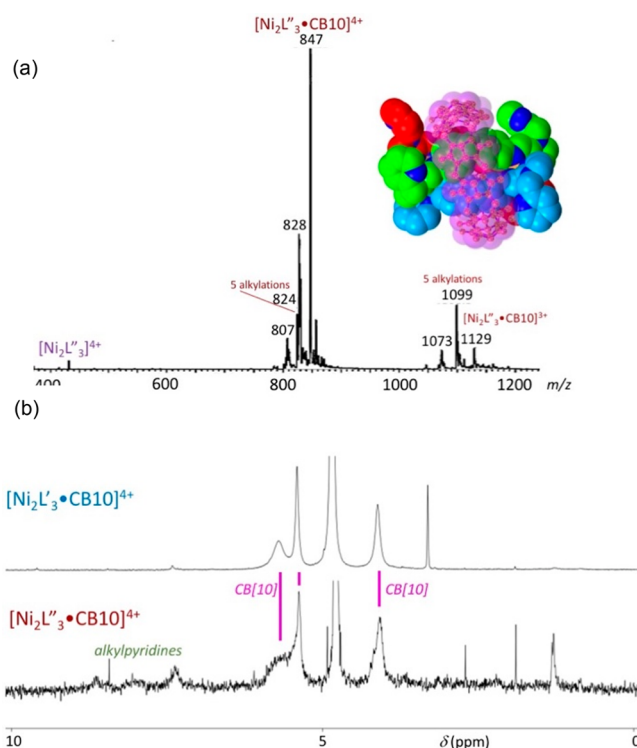


Figure 5. (a) Mass Spectrum of the $[\text{Ni}_2\text{L}''_3\cdot\text{CB10}]^{4+}$ rotaxane (b) ^1H NMR Spectrum (0–10 ppm shown only) of the $[\text{Ni}_2\text{L}''_3\cdot\text{CB10}]^{4+}$ rotaxane and comparison with imidazole pseudo-rotaxane $[\text{Ni}_2\text{L}'_3\cdot\text{CB10}]^{4+}$ showing presence of CB[10] and the alkylpyridine groups on the modified cylinder.

is formed in which the ring is now constrained by the picoyl groups to remain on the cylinder.

The (paramagnetically shifted) proton NMR spectrum shows the presence of peaks corresponding to the cylinder and the CB[10] (Figures 5 and S13). The cylinder peaks in the rotaxane are further broadened than those observed in the free $[\text{Ni}_2\text{L}''_3]^{4+}$ cylinder; consistent with this, MD simulations (Figures 4 and 6) indicate that the picoyl groups restrict the spinning motion of the cylinder about the metal–metal axis while rotaxanated inside the CB[10]. The rotaxanated complex $[\text{Ni}_2\text{L}''_3\cdot\text{CB10}]^{4+}$ was mixed with an equimolar amount of $[\text{Fe}_2\text{L}_3]^{4+}$ and heated under reflux for 24 h in 10% methanol/water. The rotaxane was unchanged and no Fe_2L_3 pseudo-rotaxanes were observed by mass spectrometry; this contrasts with the analogous mixing experiments with pseudo-rotaxanes and is strong evidence of proper rotaxane formation.

Rotaxanation would also be expected to enhance the inherent stability of the cylinder and to explore this we treated the picoyl functionalized cylinder $[\text{Ni}_2\text{L}''_3]^{4+}$ and the rotaxane $[\text{Ni}_2\text{L}''_3\cdot\text{CB10}]^{4+}$ with EDTA. EDTA binds strongly to Ni^{2+} in water ($\log K > 18$)⁶⁸ and is able to remove nickel from $[\text{Ni}_2\text{L}''_3]^{4+}$ over a period of hours at room temperature. The rotaxanation does provides additional stabilization, and the rotaxanated cylinder is competitive with EDTA for nickel binding (Figure S21) with significant amounts of rotaxane still present even after treatment with 10 equiv of $\text{Na}_2(\text{EDTA})$.

Multiply-threaded rotaxanes are structures of considerable interest.^{69–71} Considering only the ligands of the helicate the rotaxane might be viewed as a [4]rotaxane with two metal bound to the three strands. However, mass spectrometric analysis demonstrates that on demetallation the ligands are no

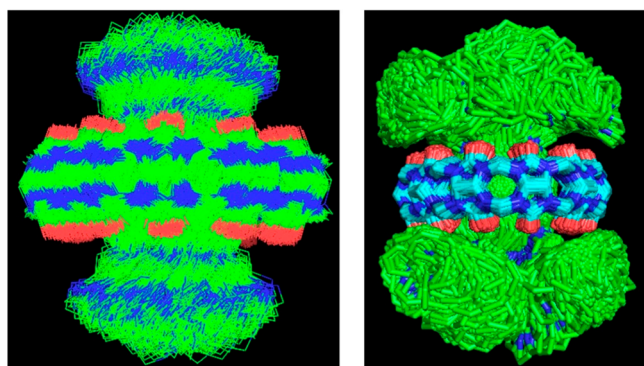


Figure 6. Combined and superimposed views of Molecular Dynamics simulations of the pseudo-rotaxanated $[\text{Ni}_2\text{L}'_3 \cdot \text{CB10}]^{4+}$ (left) and proper rotaxanated $[\text{Ni}_2\text{L}''_3 \cdot \text{CB10}]^{4+}$ (right) complexes showing the free rotation of the $[\text{Ni}_2\text{L}'_3]^{4+}$ cation in the CB[10] and the more restricted motion caused by the picolyl groups for $[\text{Ni}_2\text{L}''_3]^{4+}$; in the 1 μs time scale the rotaxanated cylinder does not rotate. Hydrogens are omitted for clarity. Supplementary videos of the simulations are also available. There is a tendency for the CB[10] ring to undergo fluxional distortions during the simulation, including a buckling of the ring at the CH_2 groups that create a heart-shaped ring and allowing it to close up around the cylinder. This is also seen at very low frequency ($<0.1\%$) in simulations of the free CB[10] but is more prevalent in the rotaxane. The paramagnetic broadening in the ^1H NMR does not allow this feature to be confirmed experimentally but reflects the greater flexibility of CB[10] compared to lower order cucubit[n]urils. See also Figures S18 and S25.

longer sterically constrained in the CB[10] cavity and dethread (Figure S21). The metallo-supramolecular assembly of the triple-helicate is thus crucial to the design and rotaxane preparation, and we consider a [2]rotaxane nomenclature to be more appropriate for this particular supramolecule, though it might be possible to access [4]rotaxanes by this approach in the future using bulkier groups.

The *non-rotaxanated* $[\text{Ni}_2\text{L}'_3]^{4+}$ and $[\text{Ni}_2\text{L}''_3]^{4+}$ cylinders bind DNA analogously to the original pyridyl cylinders (Figures 7, S16, and S17). PAGE gel electrophoresis experiments confirm that both are able to bind and stabilize DNA 3-way junctions, just as the original pyridyl cylinders do, with $[\text{Ni}_2\text{L}'_3]^{4+}$ less effective than the parent (perhaps due to imidazole deprotonation) and $[\text{Ni}_2\text{L}''_3]^{4+}$ slightly more so (perhaps because of its larger surfaces which extend into the 3WJ grooves—Figure S22). Circular dichroism spectra confirm that both cylinders bind calf thymus DNA (inducing a CD signal in the cylinder spectroscopy) which retains a B-DNA structure, and the linear dichroism demonstrates that both cylinders give rise to DNA-coiling as observed with pyridyl-based cylinders. Cell proliferation (MTT) assays confirmed that the functionalized (but non-rotaxanated) cylinder $[\text{Ni}_2\text{L}''_3]^{4+}$ exerts a cytotoxic effect in cells (IC_{50} , 72 h, MDA-MB-231 $55 \pm 10 \mu\text{M}$; SKOV3 $52 \pm 5 \mu\text{M}$). Thus, as anticipated, making changes to the ends of these cylinders does not, of itself, prevent the key DNA-binding and biological activities.

The contrast with the *rotaxane* $[\text{Ni}_2\text{L}''_3 \cdot \text{CB10}]^{4+}$ is striking (Figure 7). The rotaxane can no longer stabilize the DNA 3WJ in the gel electrophoresis experiment. It also no longer coils DNA (rather a small increase in DNA orientation is observed). Thus, wrapping the CB[10] macrocycle around the central phenylene units has switched off the DNA junction binding and DNA coiling as anticipated. The greater DNA orientation

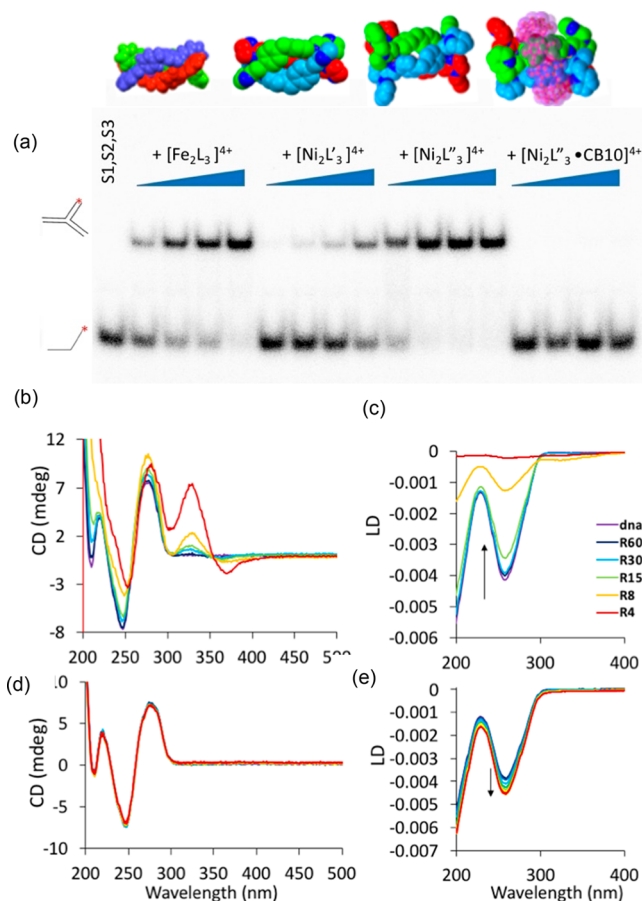


Figure 7. (a) PAGE gel showing that the $[\text{Ni}_2\text{L}'_3]^{4+}$ and $[\text{Ni}_2\text{L}''_3]^{4+}$ complexes stabilize DNA 3WJ but the rotaxanated $[\text{Ni}_2\text{L}''_3 \cdot \text{CB10}]^{4+}$ cylinder does not. (b) CD and (c) LD spectra of ct-DNA (100 μM DNA concentration; 10 mM sodium chloride; 1 mM sodium cacodylate) with $[\text{Ni}_2\text{L}'_3]^{4+}$ and (d) the corresponding CD and (e) LD spectra of ct-DNA with $[\text{Ni}_2\text{L}''_3 \cdot \text{CB10}]^{4+}$. R values represent DNA base pairs/complex.

observed in a Couette cell in the linear dichroism experiment indicates some form of DNA binding occurs, while the circular dichroism confirms that the DNA remains in its B form. Molecular dynamics simulations (Figure S18) suggest that the rotaxane may associate through a partial entry into the grooves, and that this leads to some small elongation of the DNA. Association with the bases at the ends of the DNA strand is also seen in the simulations together with some capacity to bridge across two pieces of DNA that could also lead to enhanced orientation in a Couette cell.

Just as the rotaxation switches off the key DNA binding features of the cylinders, it also switches off the biological action with the rotaxane showing no cytotoxic effect in cell proliferation (MTT) assays (IC_{50} , 72 h $\gg 140 \mu\text{M}$) in MDA-MB-231 (breast) cell lines (Figure S11).

To explore thread release we prepared a rotaxane mixture in which we only partially alkylated the rotaxane, thereby varying the number of picolyl branches/stoppers on the species, and creating a mixture of rotaxanes containing 3-, 4-, and 5-stoppers on the thread as well as the fully-stoppered 6-alkylated rotaxane (Figure 8). We then undertook a competition experiment with the $[\text{Fe}_2\text{L}_3]^{4+}$ cylinder (Figures 8 and S23).

The results are consistent with a kinetic de-rotaxanation for cylinders that are not fully stoppered: the competitor initially

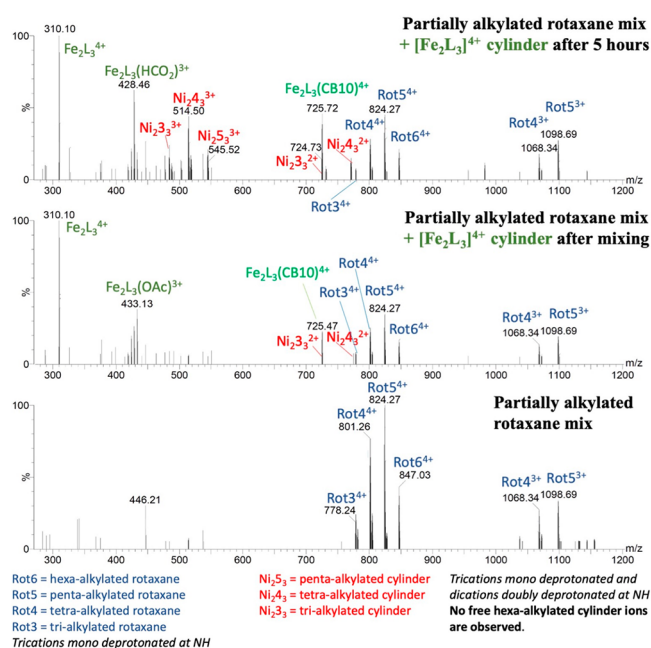


Figure 8. Electrospray mass spectra of a competition experiment treating a partially alkylated rotaxane mix $[\text{Ni}_2\text{L}''_3\text{CB10}]^{4+}$ with 1 equiv of $[\text{Fe}_2\text{L}_3]^{4+}$ in aqueous methanol (1:1) showing the dethreading of partially alkylated cylinders and the formation of pseudo-rotaxane $[\text{Fe}_2\text{L}_3\text{CB10}]^{4+}$. No peaks corresponding to dethreading of fully alkylated (hexa-alkylated) cylinders were observed. Figure S23 shows further timepoints.

displaces tri- and tetra-alkylated cylinders from the CB[10] ring and after further time also penta-alkylated cylinders. No peaks corresponding to displacement of hexa-alkylated (fully alkylated) cylinders are observed even over long time periods (28 h). An analogous experiment with 5 and 10 equiv of competitor guest memantine (Figure S24a) showed similar effects.

The tri-alkylated cylinders, at one end bear just one or no stoppers, and the tetra-alkylated cylinders have an end with either one or two stoppers. The penta-alkylated cylinder has one end with two stoppers, while in the hexa-alkylated cylinder both ends contain three stoppers. The results imply that cylinders with a mono-alkylated end de-thread more readily than those whose ends are di-alkylated, consistent with the greater steric barrier to de-threading that increased alkylation should present.

This poorly alkylated rotaxane mixture also gave rise to a weak 3WJ band in gel electrophoresis (Figure S24b), consistent with a small proportion of poorly alkylated cylinders dethreading from the CB[10] and binding instead to the 3WJ. These results provide intriguing insight into how the extent and type of branch-point alkylation might be used to control the kinetics of de-rotaxation and in turn kinetically and temporally modulate release of the 3WJ-binding drug.

CONCLUSIONS

We have demonstrated a new approach to the design of rotaxanes by employing cylindrical metallo-supramolecular helicates as the axle, with the ring held in place by branch points which emanate from the strands of the helix. These intriguing new agents are assembled by combining two designs on which the field of supramolecular chemistry was founded: the metallo-helicates (pioneered by Lehn)⁹ and rotaxanes

(Stoddart and Sauvage).^{1,2} The metallo-cylinders (a class of triple-helicates) used as the rotaxane axle, offer unique and unprecedented DNA binding properties; their activity has stimulated a great interest in designing metallo-supramolecular drugs.^{11,12} Pseudo-rotaxation is not sufficient to modify the bio-activity of these cylinders in vitro or in cellulo, but by proper rotaxation those properties are modified.

Switching the activity of a metallo-drug on rotaxation represents an unconventional approach: indeed incorporation of metallo-drugs into proper rotaxanes is rare and restricted to mononuclear platinum⁷² and ruthenium⁷³ drugs attached to the terminal stopper of a rotaxane, with the rotaxane part essentially behaving as an attached organic vector. Organic drugs such as paclitaxel have also been attached as a rotaxane stopper,⁷⁴ while Papot and Leigh have used a potentially bioactive peptide as a linear thread in a hydrogen-bond assembled rotaxane and combined it with a sophisticated enzyme-cleavage rotaxane degradative release.^{75,76}

Beyond the temporal control of activity that we explore, our results also raise the tantalizing possibility of creating systems in which a triggered rotaxation or de-rotaxation (de-threading) inside a cell might be used to switch a metallo-drug action on or off. This might be applicable to a range of different molecular and metallo-supramolecular designs and we are actively exploring the further potential of this approach.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/jacs.0c07750>.

- Experimental details and Figures S1–S25 (PDF)
- Movie of the MD simulations in Figure 6 (MP4)
- Movie of the MD simulations in Figure 6 (MP4)
- Crystallographic file for $[\text{Ni}_2\text{L}''_3][\text{PF}_6]_4$ (CIF)
- Crystallographic file for $[\text{Fe}_2\text{L}_3][\text{PF}_6]_4$ (CIF)

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Notes

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